

FROM PATHWAYS TO PEOPLE: MODELLING ALLERGIC CONTACT DERMATITIS

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UNILEVER R&D



ALLERGIC CONTACT DERMATITIS

1. Skin penetration and haptening: covalent modification of skin protein

2. Migration of Langerhans cells and dermal dendritic cells

3. Antigen processing and presentation by dendritic cells

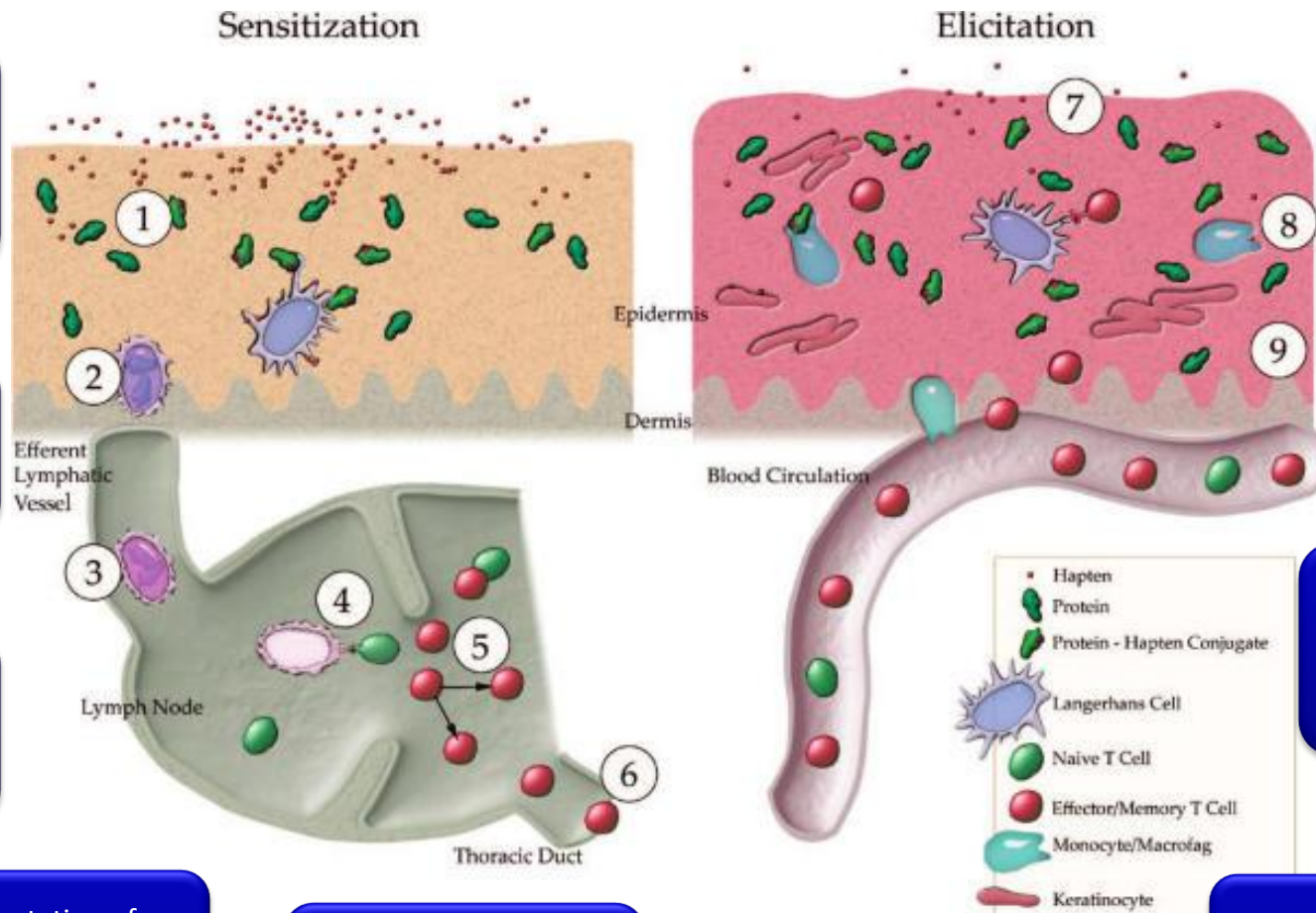
4. Presentation of haptenated peptide by dendritic cell to T cells

5. Proliferation and differentiation of specific T cells

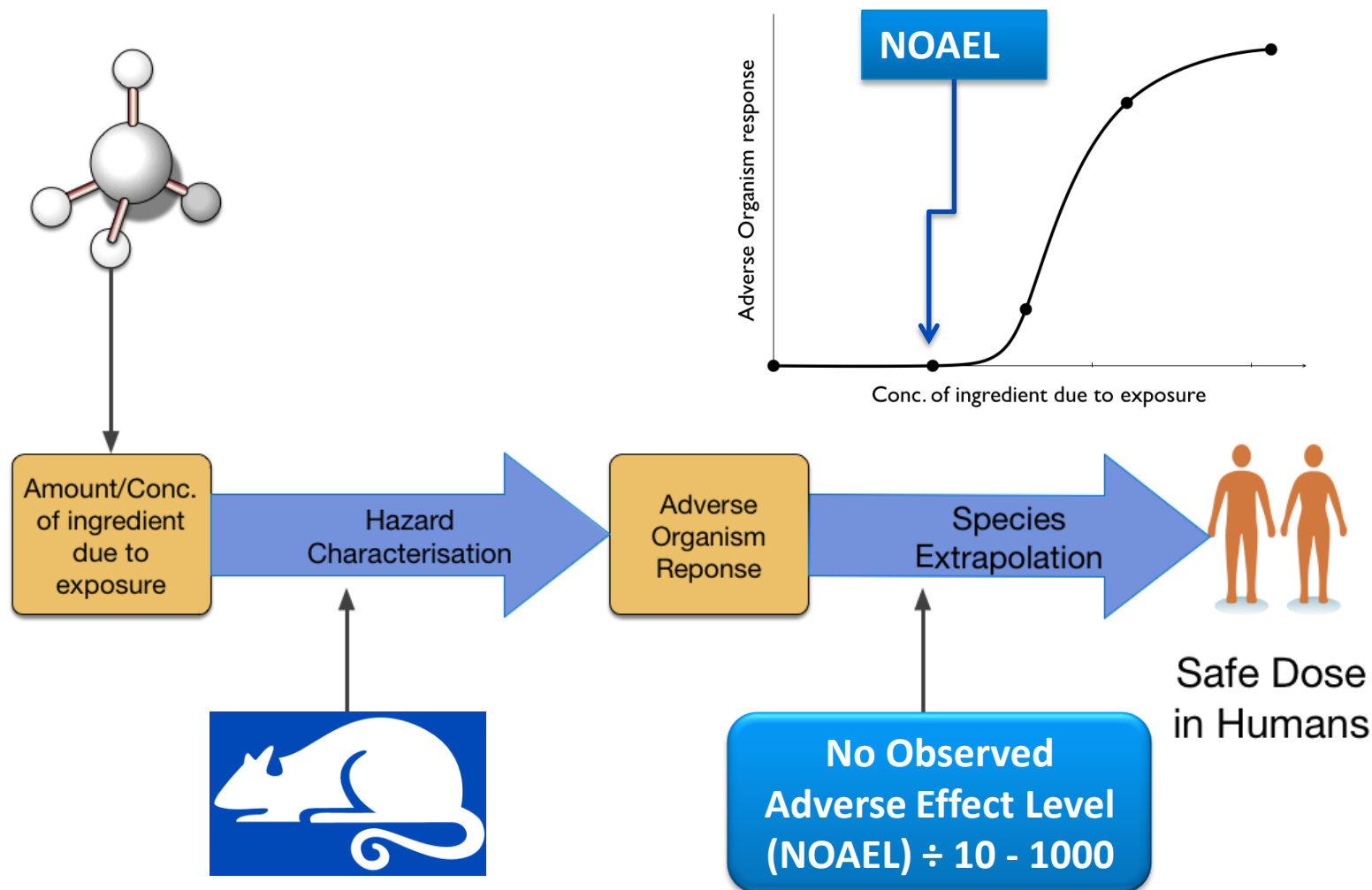
6. Generation of antigen-specific memory T cell population

7. Re-exposure to chemical

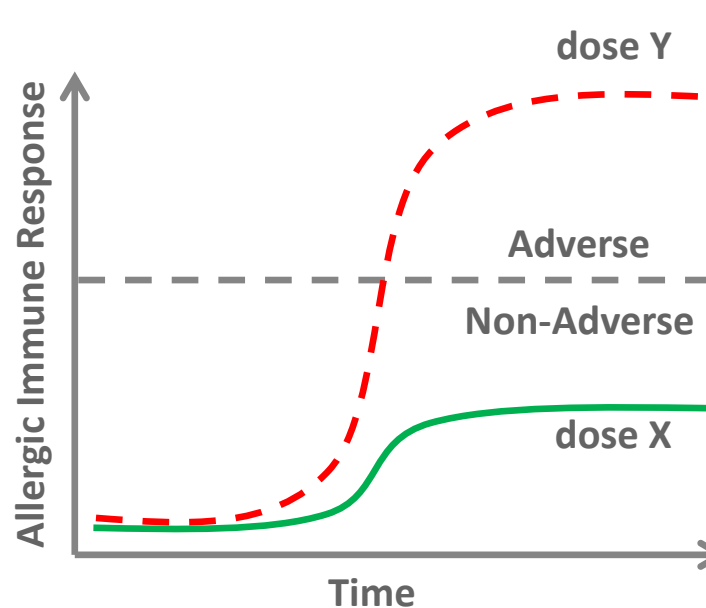
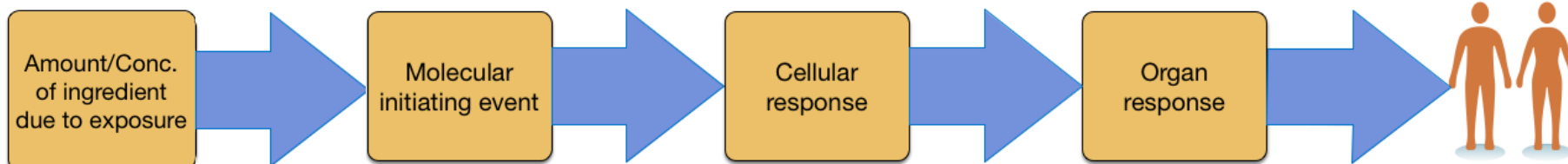
9. Recruitment of antigen-specific memory T cells and expansion of effector T cells to elicit response



CURRENT HUMAN HEALTH RISK ASSESSMENT PARADIGM FOR CHEMICAL INGREDIENTS



NEW HUMAN HEALTH RISK ASSESSMENT PARADIGM FOR SENSITISING INGREDIENTS?



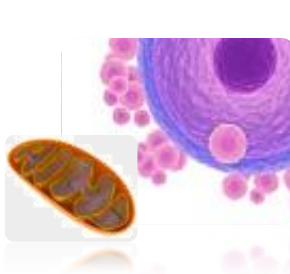
NEW HUMAN HEALTH RISK ASSESSMENT PARADIGM FOR SENSITISING INGREDIENTS?



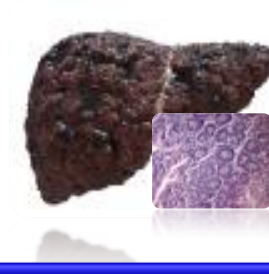
1. Skin Penetration



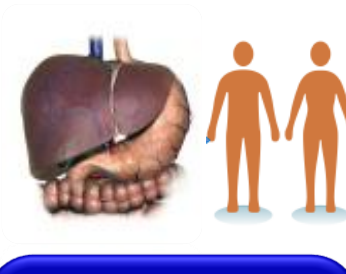
3-4. Haptenation: covalent modification of epidermal proteins



5-6. Activation of epidermal keratinocytes & Dendritic cells



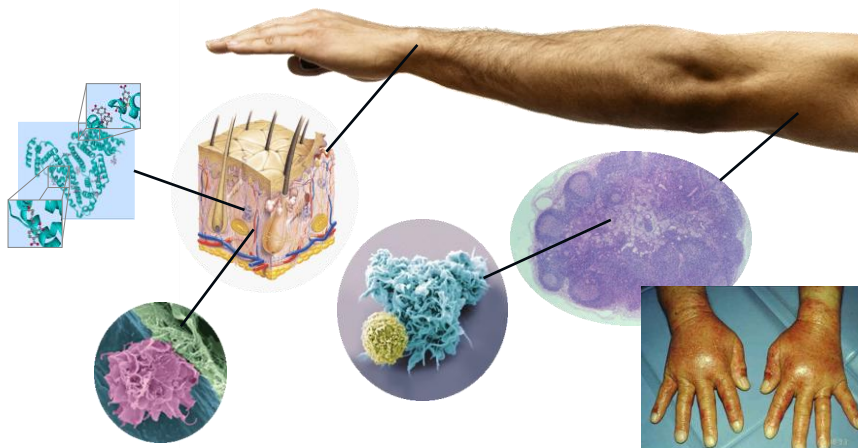
7. Presentation of haptened protein by Dendritic cell resulting in activation & proliferation of specific T cells



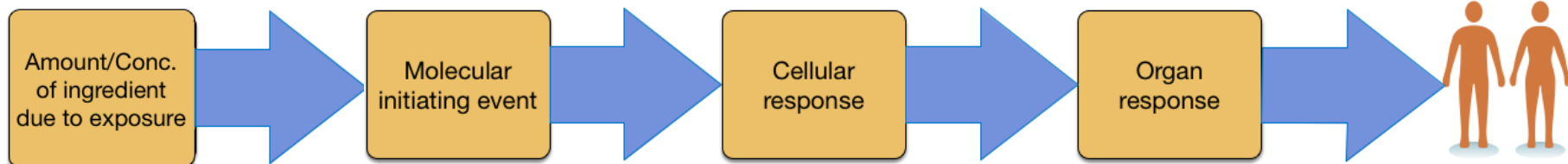
8-11. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death



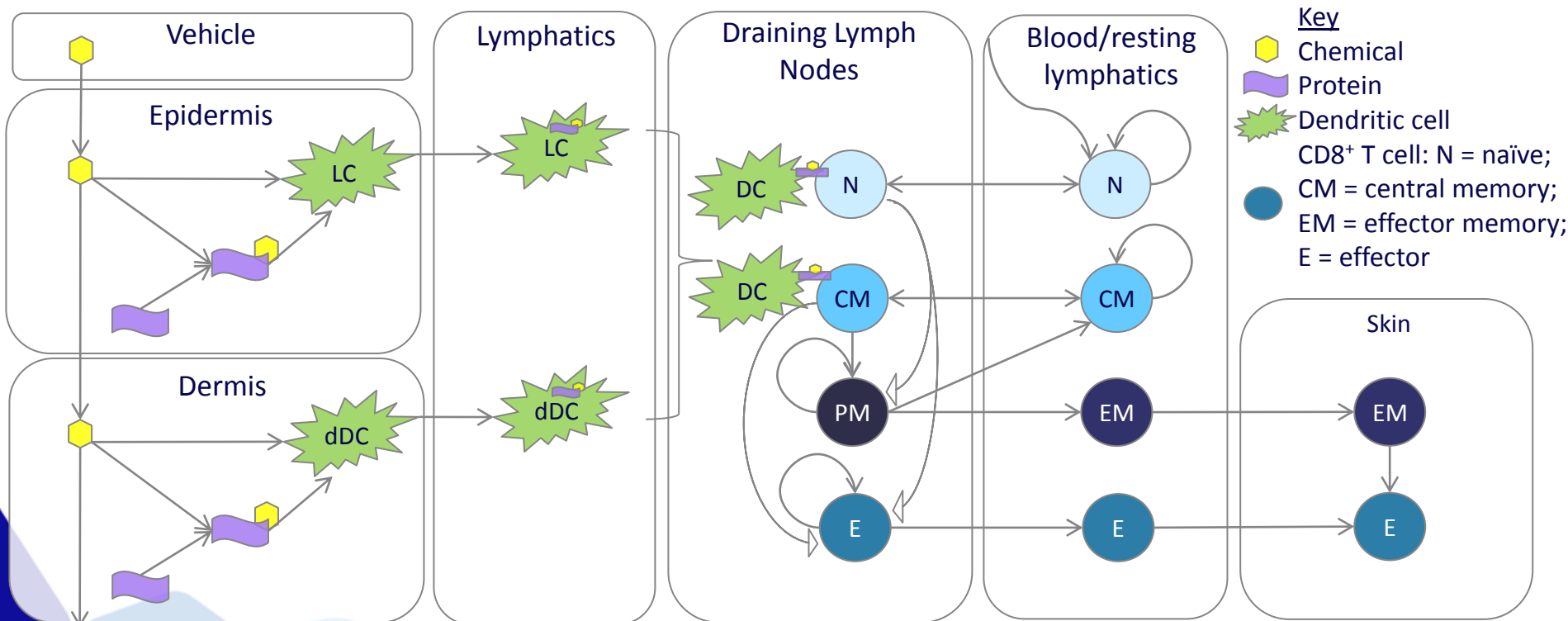
Modified from 'Adverse Outcome Pathway (AOP) for Skin Sensitisation', OECD



DEVELOP A MATHEMATICAL MODEL OF ALLERGIC CONTACT DERMATITIS TO ENABLE RISK ASSESSMENT DECISION-MAKING FOR NEW CHEMICALS



Safe Dose
in Humans



KEY ASSUMPTION: ANTIGEN DRIVING T CELL RESPONSE IS HAPTENATED PEPTIDE

Direct Acting

- haptenated residues present on pMHC initiating the response

&/or

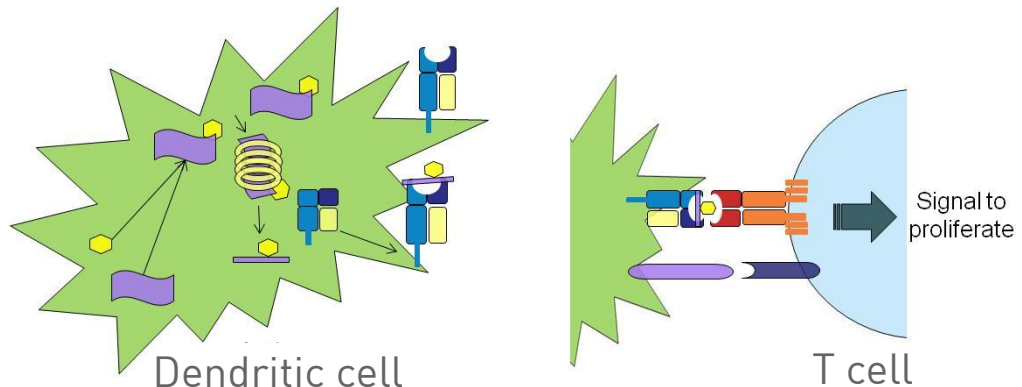
Altered Processing

- haptenated residues disrupt normal proteasome processing resulting in presentation of altered self-peptides

&/or

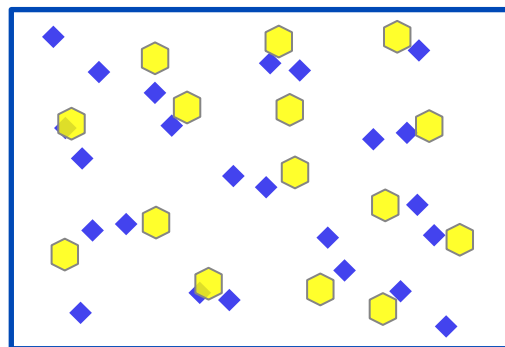
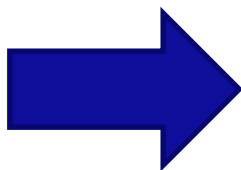
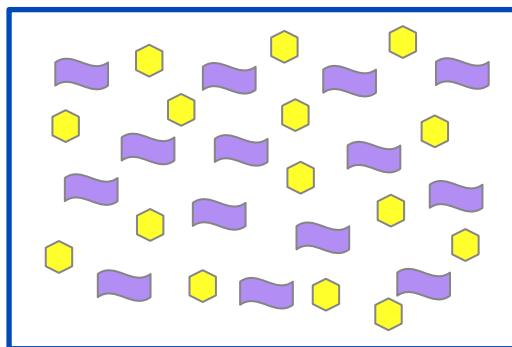
Altered Selection

- hapten activity disrupts MHC loading resulting in altered selection of self

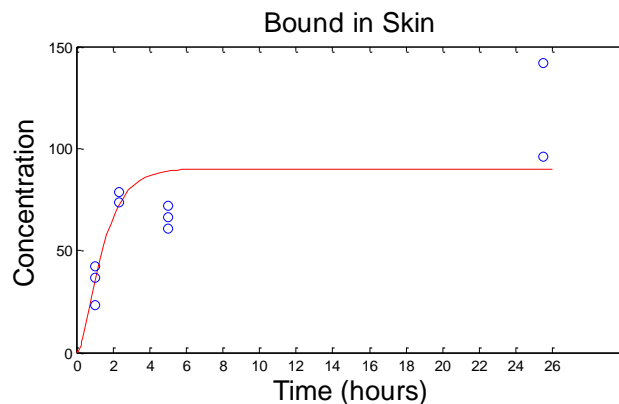
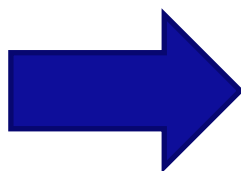
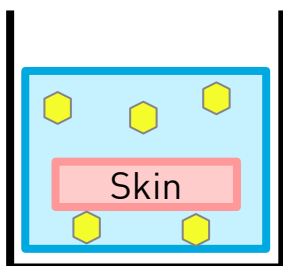





PREDICTING HAPTENATION RATE OF SKIN PROTEIN BY DI-NITROCHLOROBENZENE (DNCEB)

- Modelling approach - treat proteins as mixture of nucleophilic residues
- Use experimental data to determine 'bulk' haptenation rate & estimate the fraction of nucleophiles we expect to be haptenated

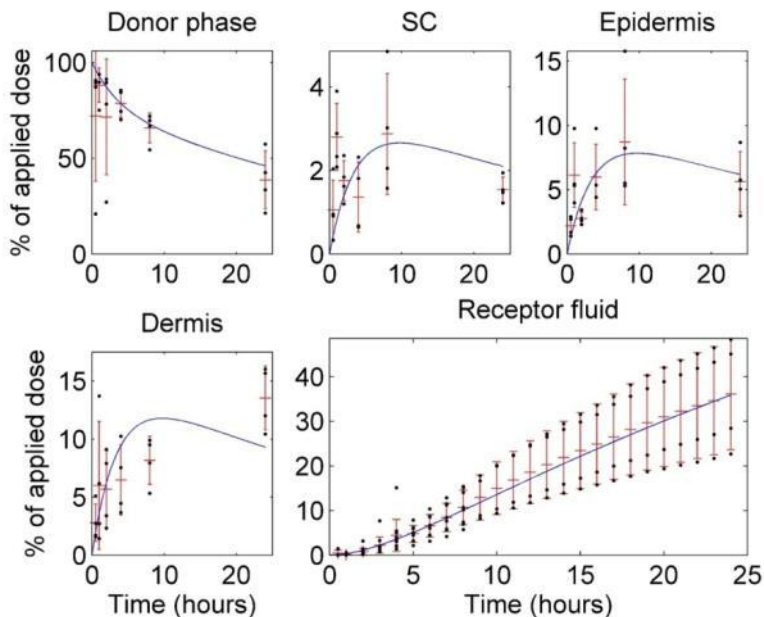
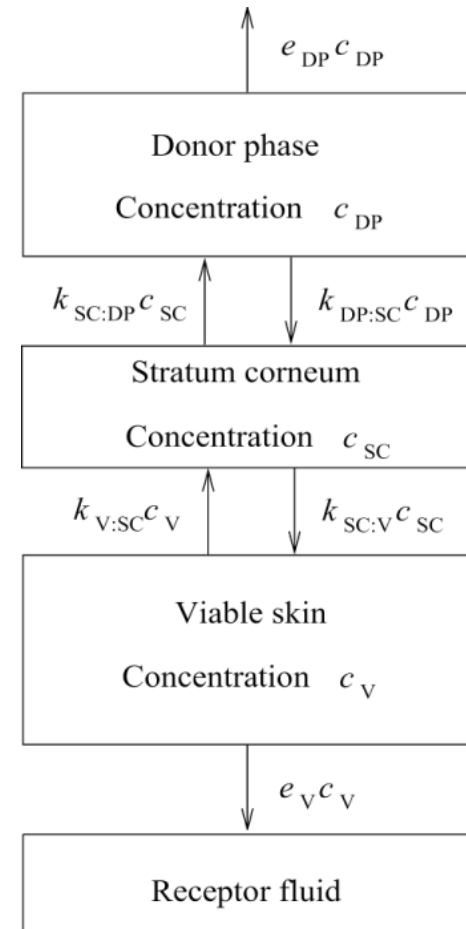
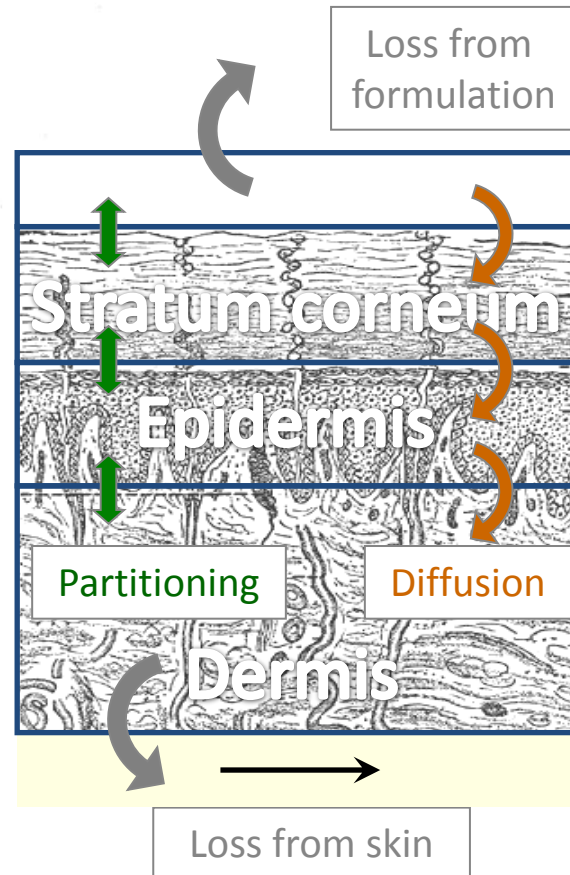
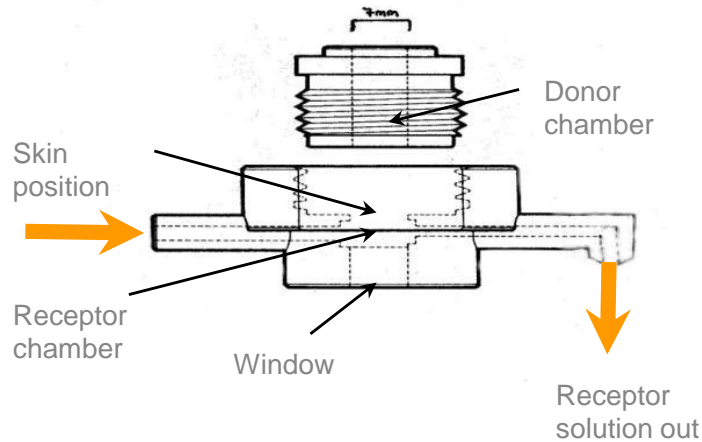


*Cys
Tyr
Lys
His
Arg
Met &
N-term*



Key
 Chemical
 Protein
 Nucleophilic residue

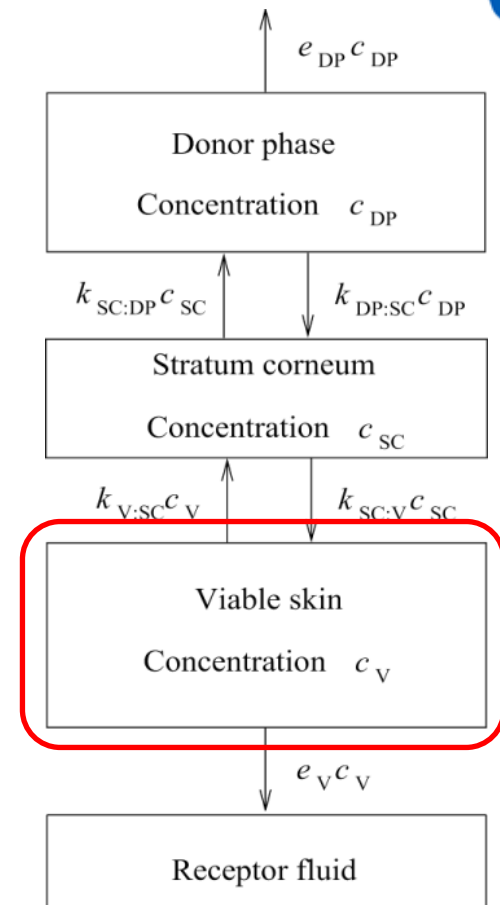
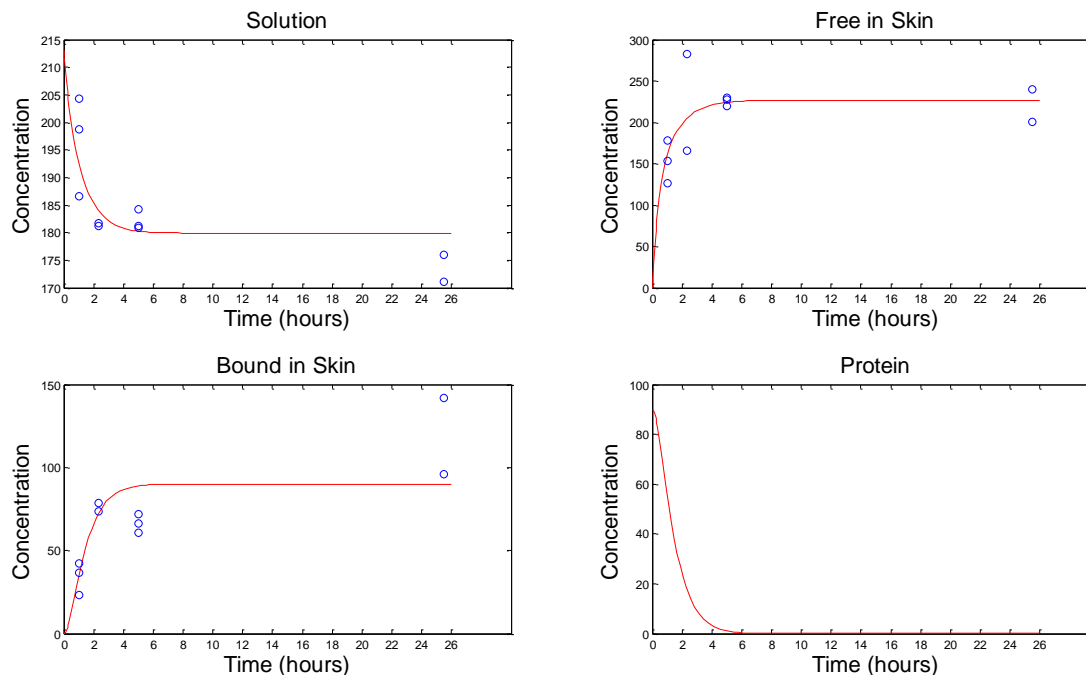
MODELLING SKIN BIOAVAILABILITY OF CHEMICAL



PREDICTING EXTENT OF SKIN PROTEIN HAPTENATION FOLLOWING SINGLE EXPOSURE TO DNCB

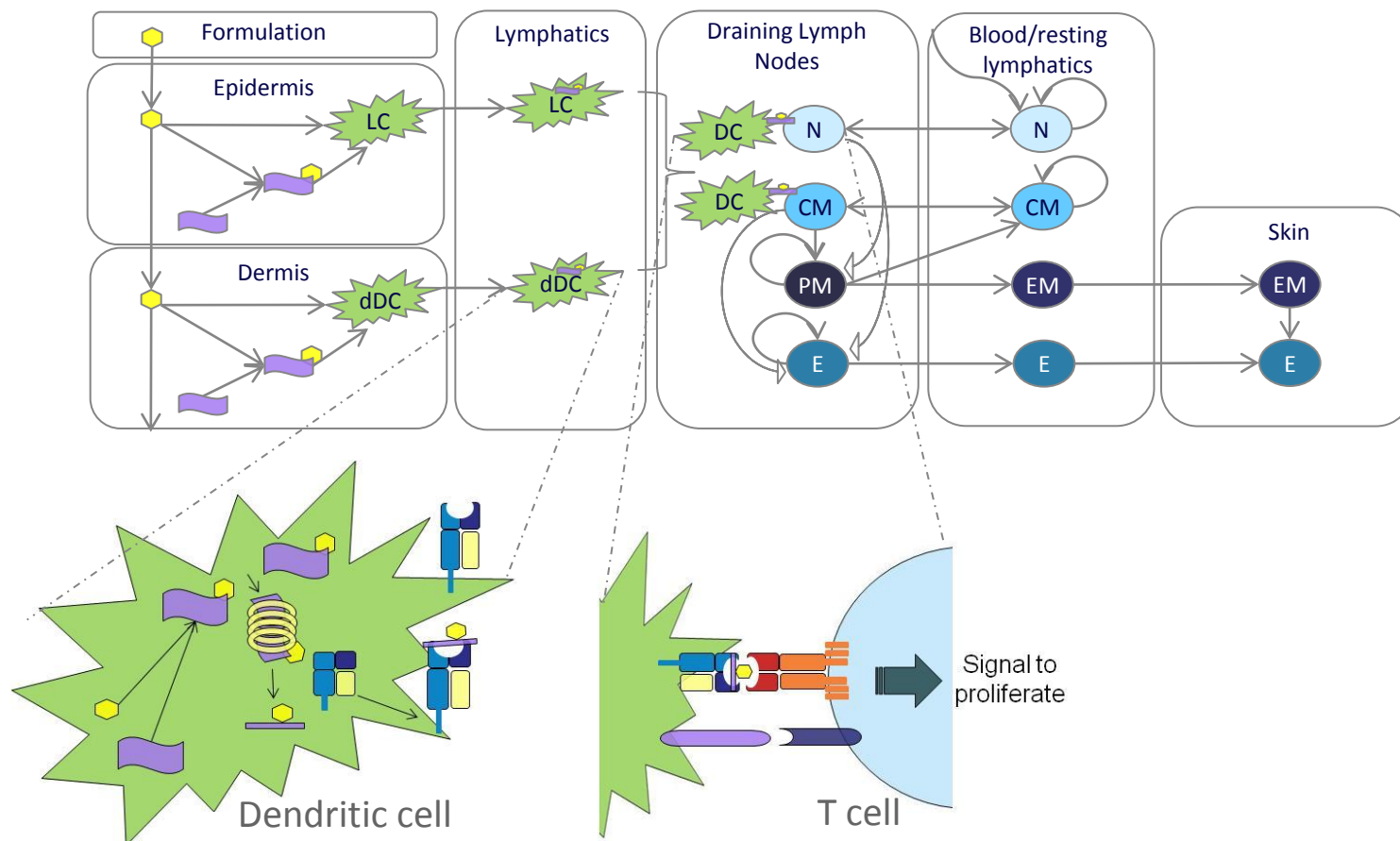
Skin bioavailability model expanded to include covalent modification of skin protein by chemical

» Amount of haptenated protein predicted over time



» Haptenated protein and free chemical concentrations are inputs to immune response model

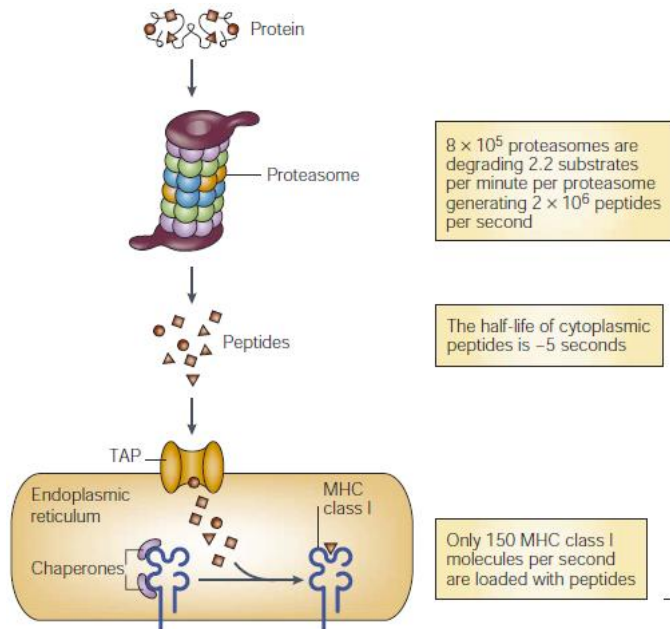
TRANSLATING CHEMICAL SENSITISER EXPOSURE INTO EXTENT OF HAPTEN PRESENTATION



- Intracellular LC/DC protein is haptenedated by free chemical
- Proteasomal processing and Class I MHC presentation
- DC-T cell synapse in draining lymph node

MODELLING PROTEASOMAL PROCESSING & CLASS I MHC ANTIGEN PRESENTATION

Assume 'Direct Acting' hypothesis (unaltered proteasomal processing) and determine properties of resulting peptides



8×10^5 proteasomes are degrading 2.2 substrates per minute per proteasome generating 2×10^6 peptides per second

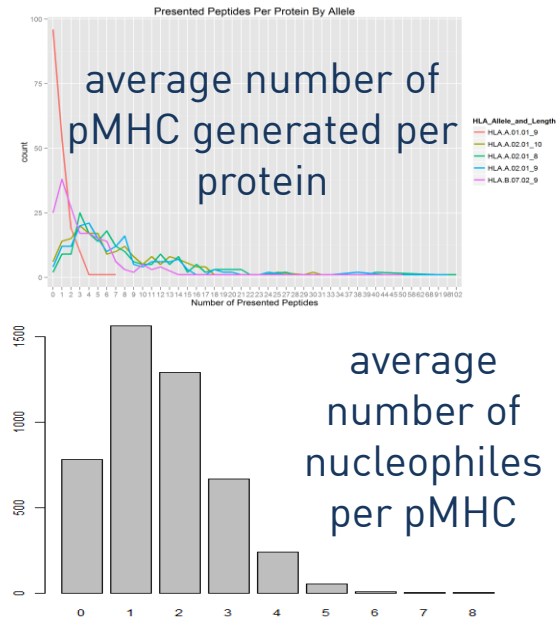
The half-life of cytoplasmic peptides is ~5 seconds

Only 150 MHC class I molecules per second are loaded with peptides

Prediction tools

Proteasomal cleavage (e.g. NetChop)

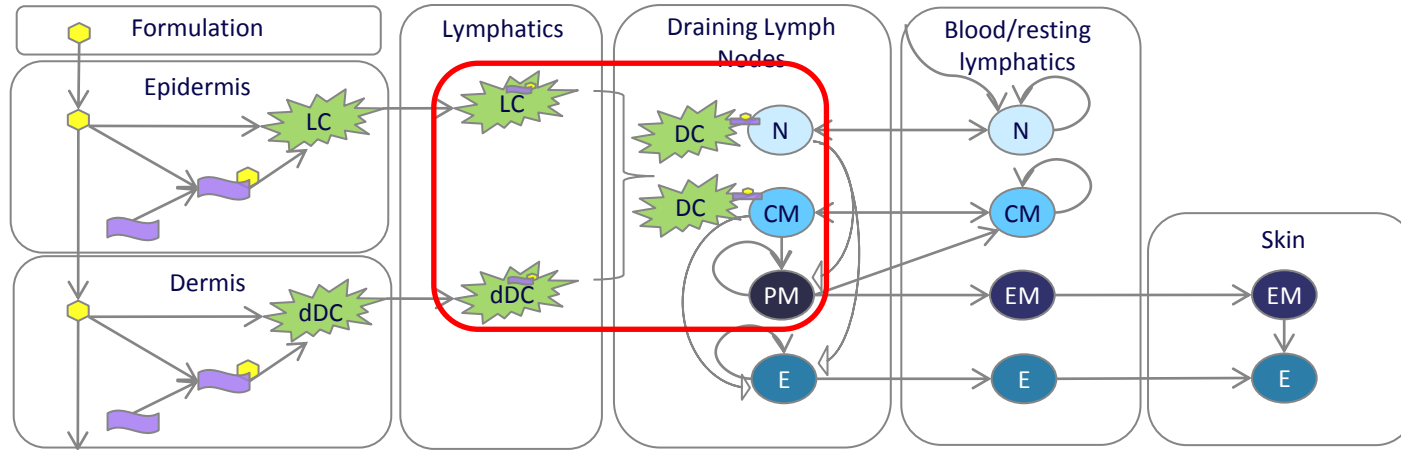
MHC I binding (e.g. NetMHCpan)



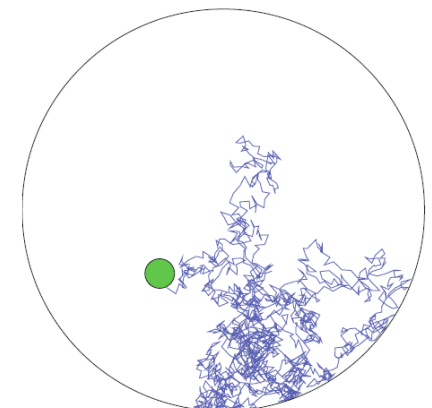
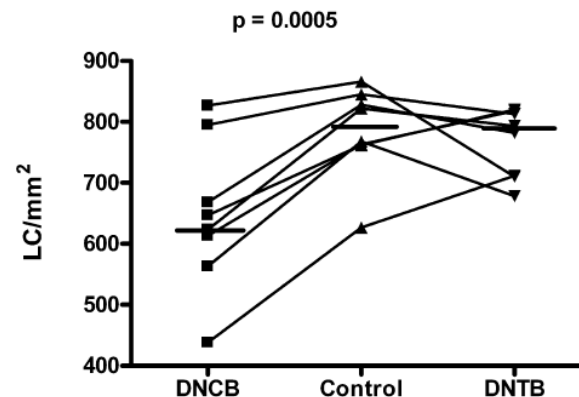
Estimate average pMHC surface density from considerations of:

1. the fraction of nucleophiles we expect to be haptenated
2. probability that a pMHC contains a haptenated nucleophile

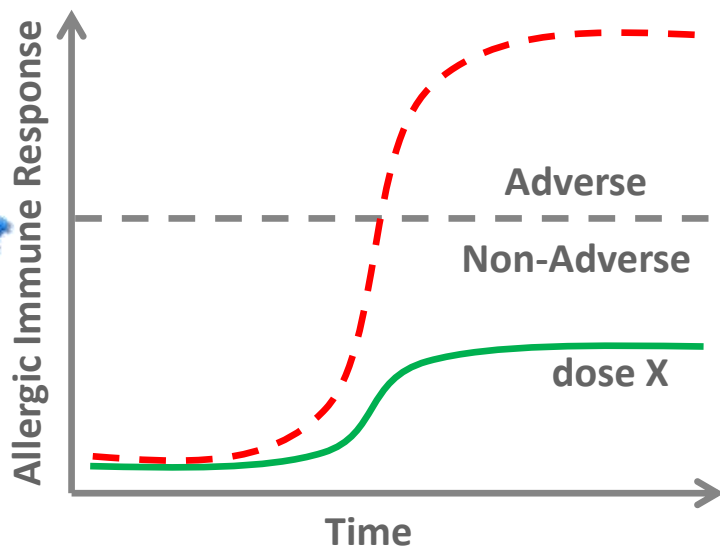
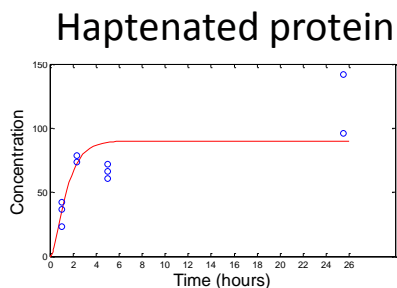
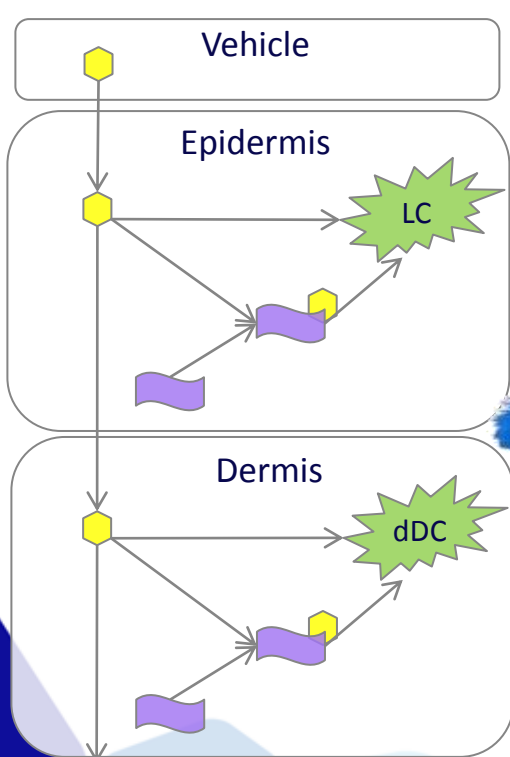
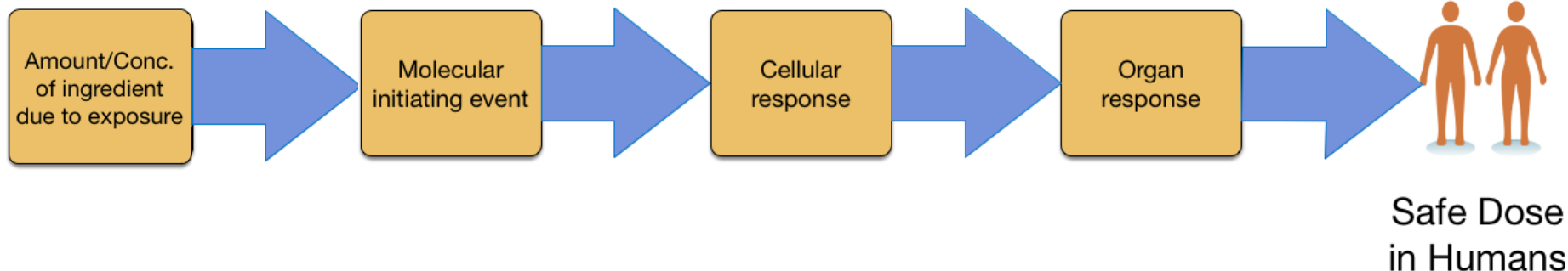
MODELLING DC-T CELL INTERACTIONS IN DRAINING LYMPH NODE



- LC/dDC migrate from sensitiser-exposed skin to present haptenated peptides via Class I MHC to $CD8^+$ T cell in draining lymph node
e.g. Pickard *et al*, 2009
- DC/T cell movement in lymph node is described by random walk
e.g. Day & Lythe, 2011



DEVELOP A MATHEMATICAL MODEL OF ALLERGIC CONTACT DERMATITIS TO ENABLE RISK ASSESSMENT DECISION-MAKING FOR NEW CHEMICALS



'T LYMPHOCYTES: ORCHESTRATORS OF SKIN SENSITISATION' WORKSHOP – MAY 2010, LONDON

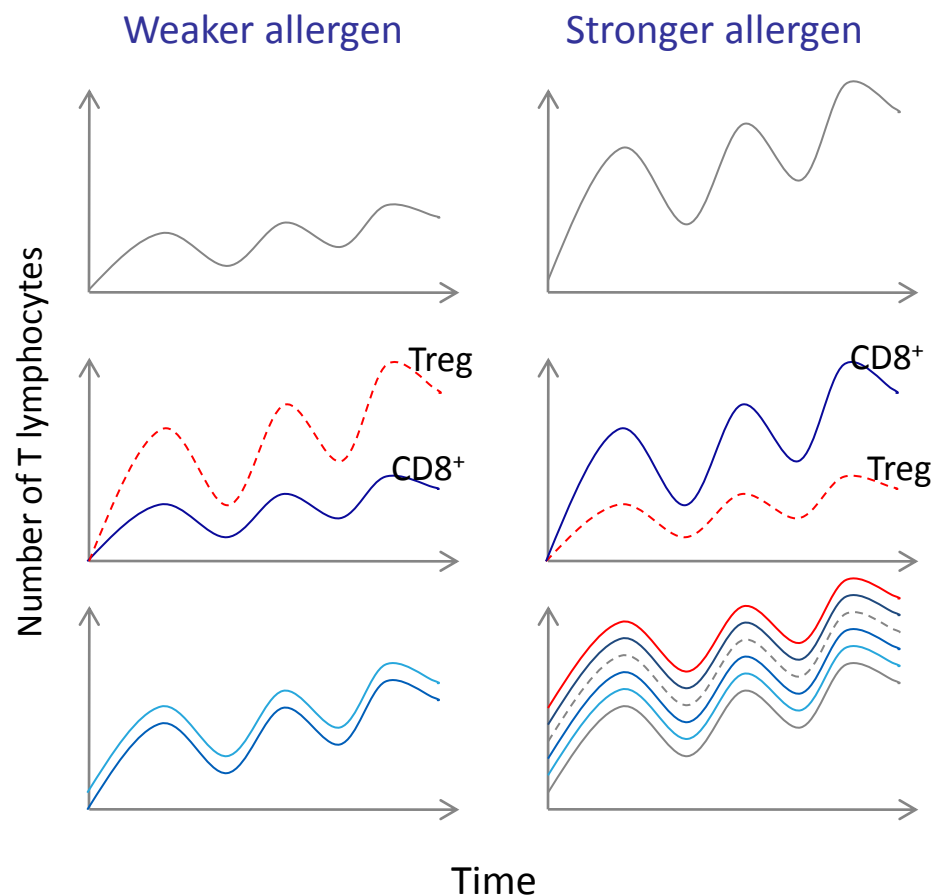


Immunologists, risk assessors & mathematical modellers – 2 day workshop

What are the characteristics of the T cell response that could reflect sensitiser potency in humans?

- » **Magnitude:** What is the extent of sensitiser-induced T cell response (volume, kinetics & duration)?
- » **Quality:** Within sensitiser-induced T cell response, what is the balance between the T cell sub-populations?
- » **Breadth:** What proportion of the T cell clonal repertoire has been stimulated by a given sensitiser?

Kimber *et al.* 2012. *Toxicology*. **291**. 18-24



CD8⁺ T CELL RESPONSE: INITIAL MODEL SCOPE

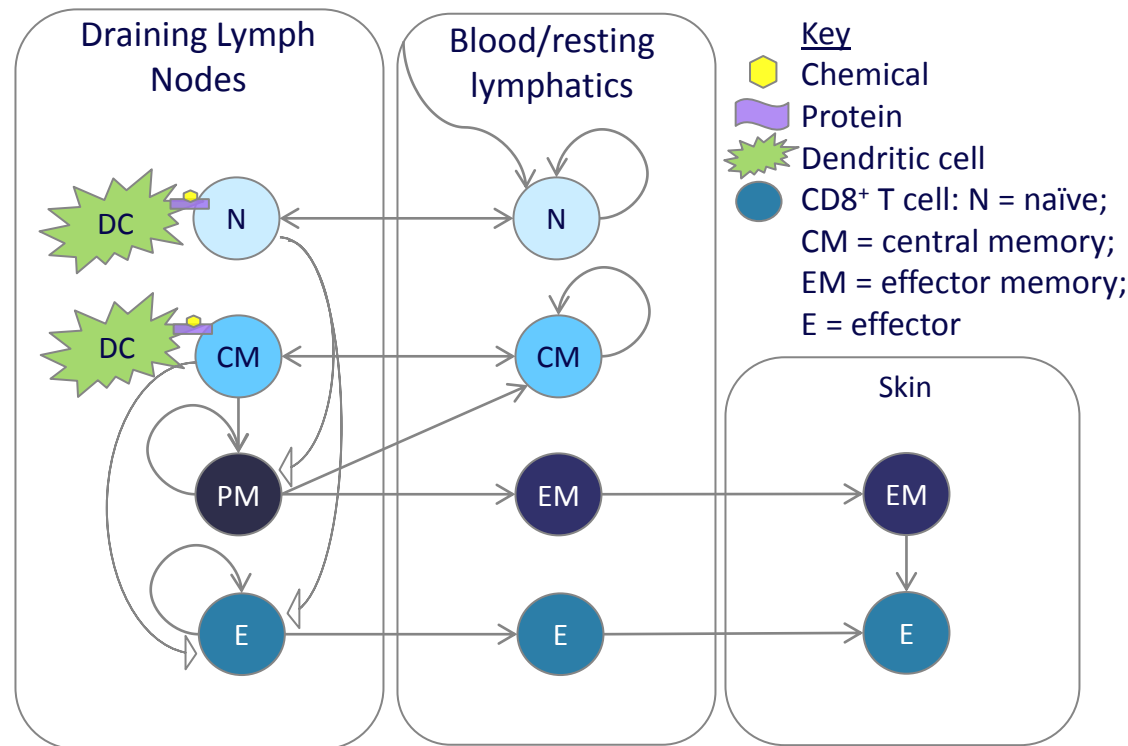
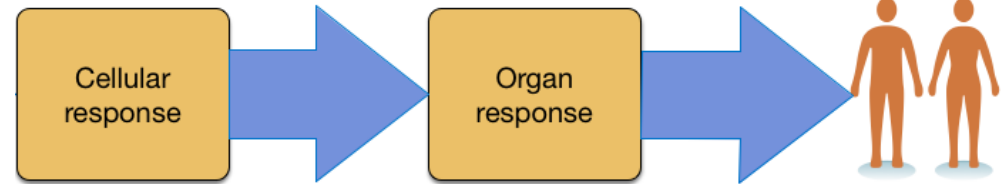


Current model scope models the antigen-specific CD8⁺ T cell response including:

- » **naïve (N)** - CD45RO^{-ve}CD62L^{+ve} or CD45RA^{+ve}CD27^{+ve}
- » **central memory (CM)** - CD45RO^{+ve}CD62L^{+ve} or CD45RA^{-ve}CD27^{+ve}
- » **effector memory (EM)** - CD45RO^{+ve}CD62L^{-ve} or CD45RA^{-ve}CD27^{-ve}
- » **effector (E)** - CD45RO^{-ve}CD62L^{-ve} or CD45RA^{+ve}CD27^{-ve}

Human sensitiser-specific T cell data is largely unavailable:

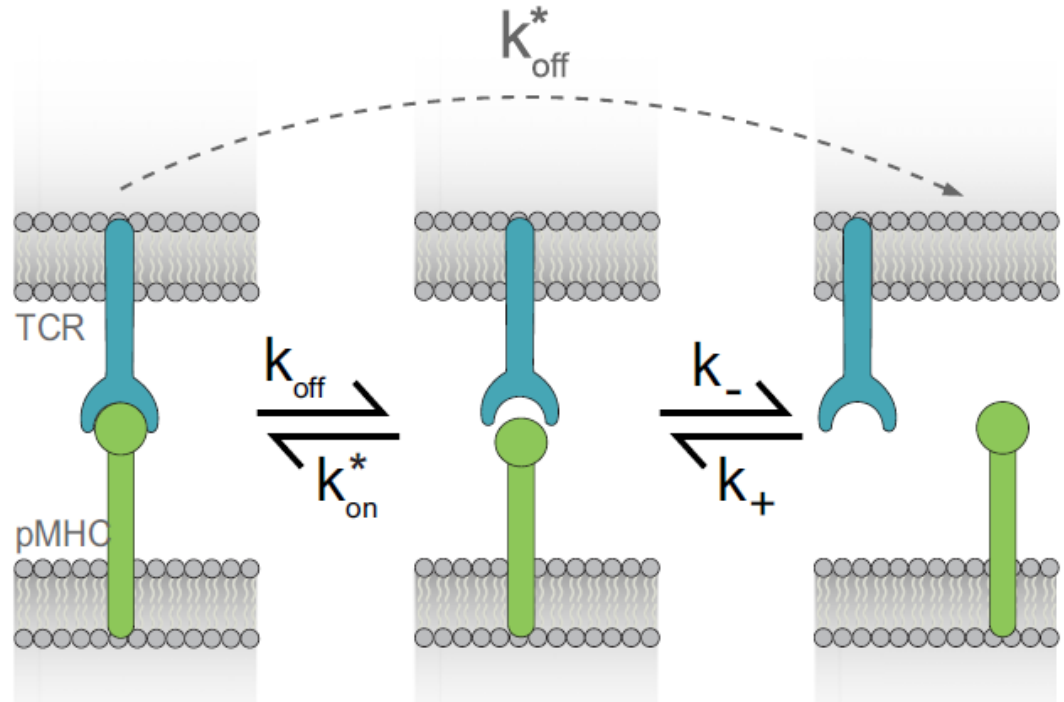
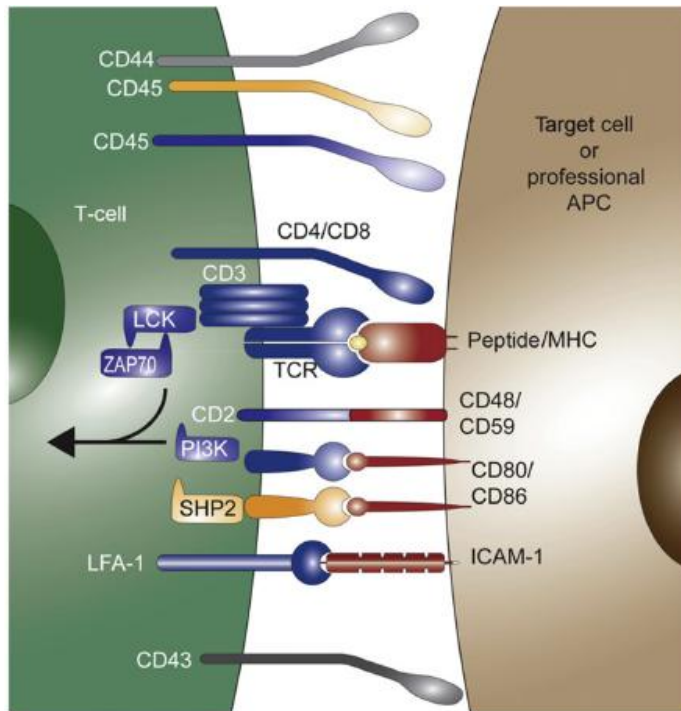
- » Make use of literature data
- » Generate sensitiser-specific, human-relevant data



PREDICTING THRESHOLD FOR T CELL ACTIVATION

Is the nature (TCR affinity) of the antigen limiting?

- what k_{on}/k_{off} do TCRs have for cognate hapten pMHC



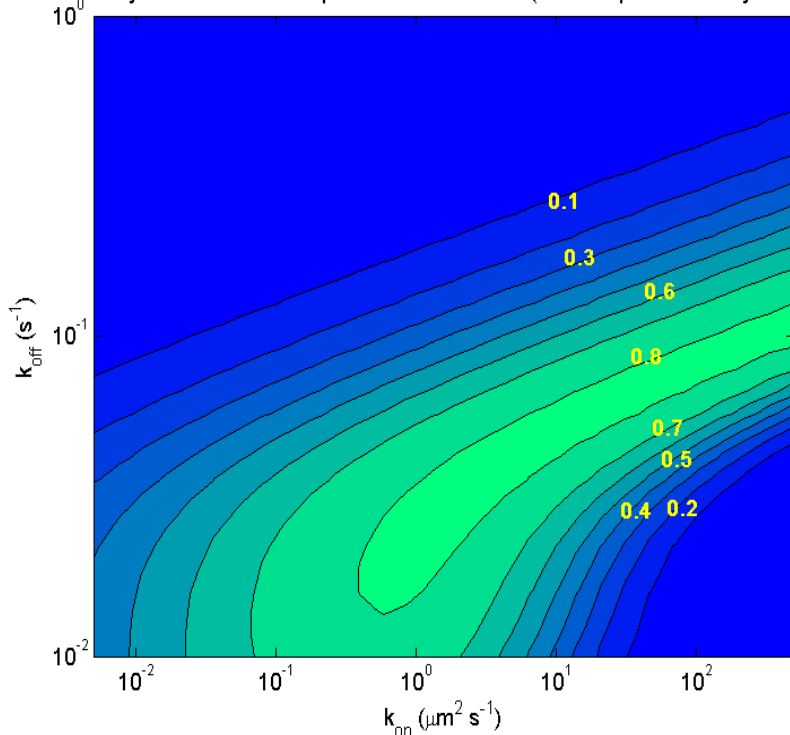
Explore effect of pMHC surface density and k_{on}/k_{off} on probability of T-cell triggering with the available models (Zarnitsyna & Zhu, 2012). Simulations generated using 'confinement time' model of Dushek, et al, 2009.

PREDICTING THRESHOLD FOR T CELL ACTIVATION

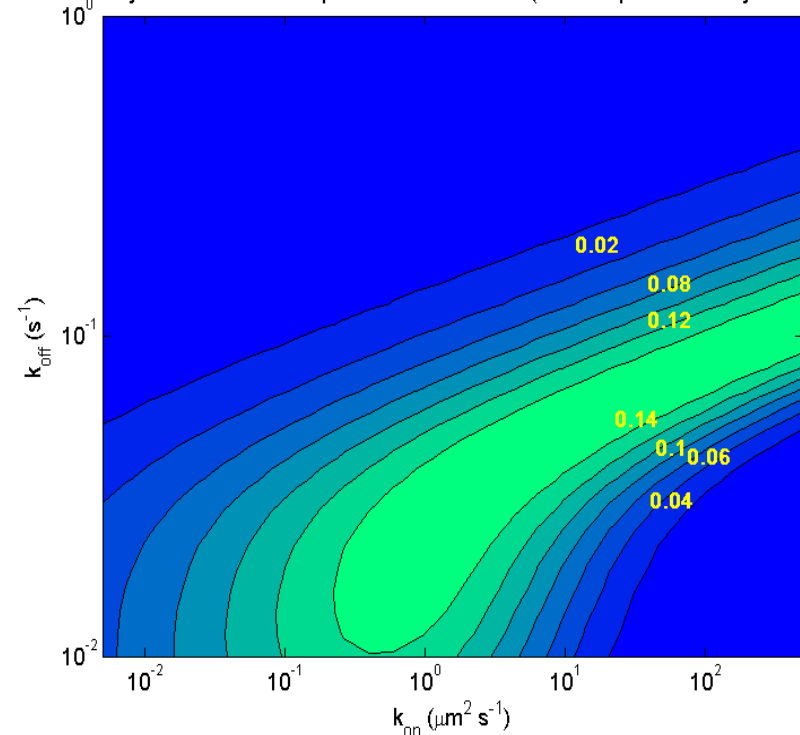
Is the nature (TCR affinity) of the antigen limiting?

- what $k_{\text{on}}/k_{\text{off}}$ do TCRs have for cognate hapten pMHC

Probability of T-cell activation per DC:T-cell contact (non-self pMHC density of 1:100)

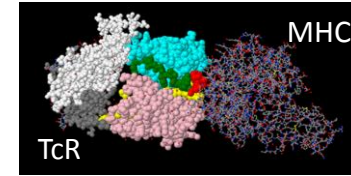


Probability of T-cell activation per DC:T-cell contact (non-self pMHC density of 1:1,000)

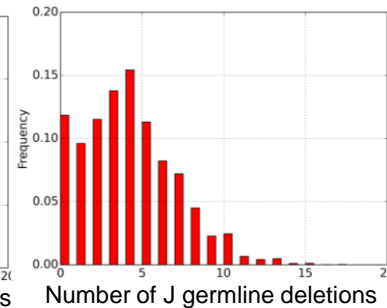
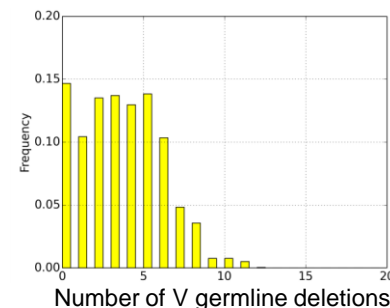
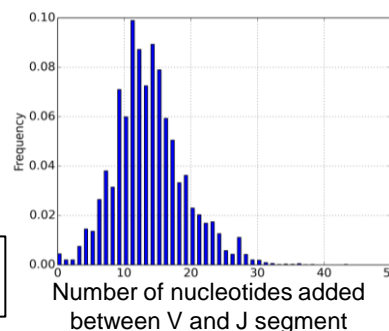
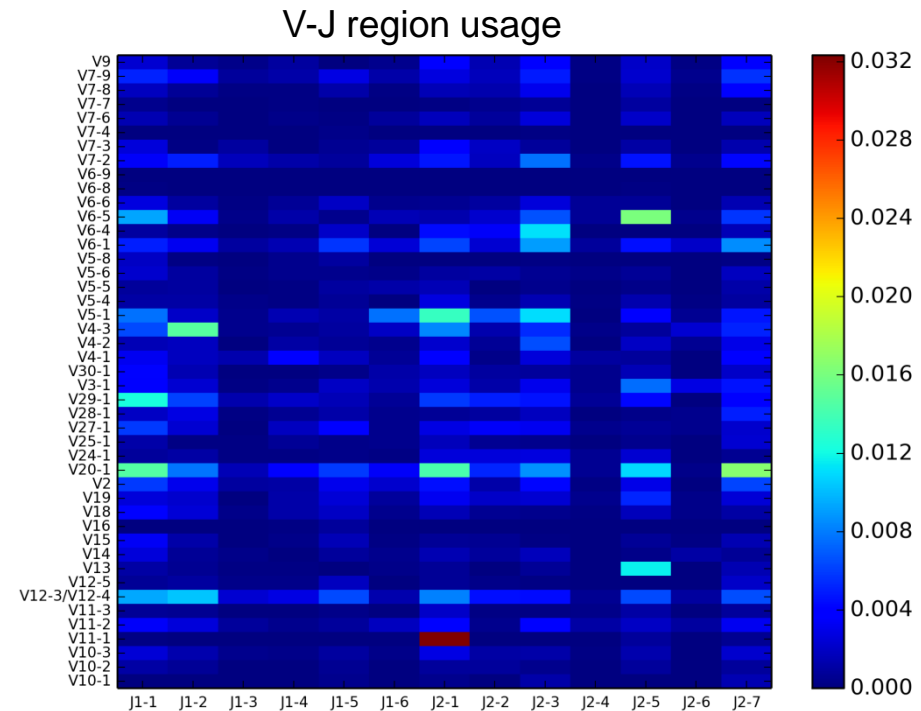


Explore effect of pMHC surface density and $k_{\text{on}}/k_{\text{off}}$ on probability of T-cell triggering with the available models (Zarnitsyna & Zhu, 2012). Simulations generated using 'confinement time' model of Dushek, et al, 2009.

Molecular basis of T cell recognition: how do TcRs interact with sensitising antigens?

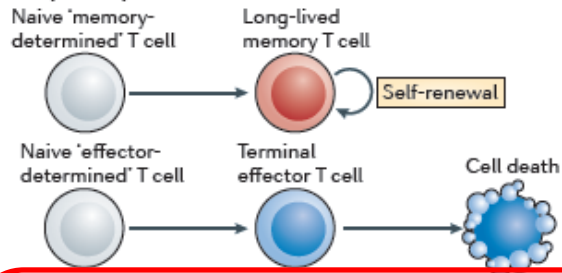


- Thermodynamic and kinetic parameters
- Role of MHC
- Characteristics of the CDR3s, and framework
- Using DeCombinatoR: (<https://github.com/uclinfectionimmunity/Decombinator>) to assign TcR sequences - V region usage, J region usage, no. of V deletions, no. of J deletions, CDR3 sequence read

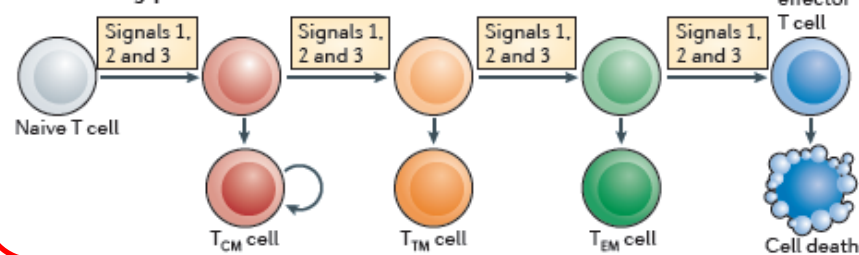


CD8+ T CELL DIFFERENTIATION: COMPARING CURRENT HYPOTHESES

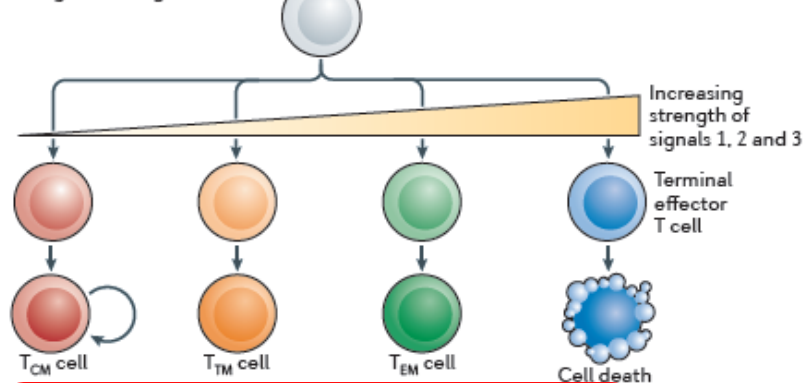
a Separate-precursor model



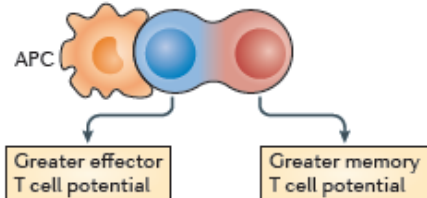
b Decreasing-potential model



c Signal-strength model



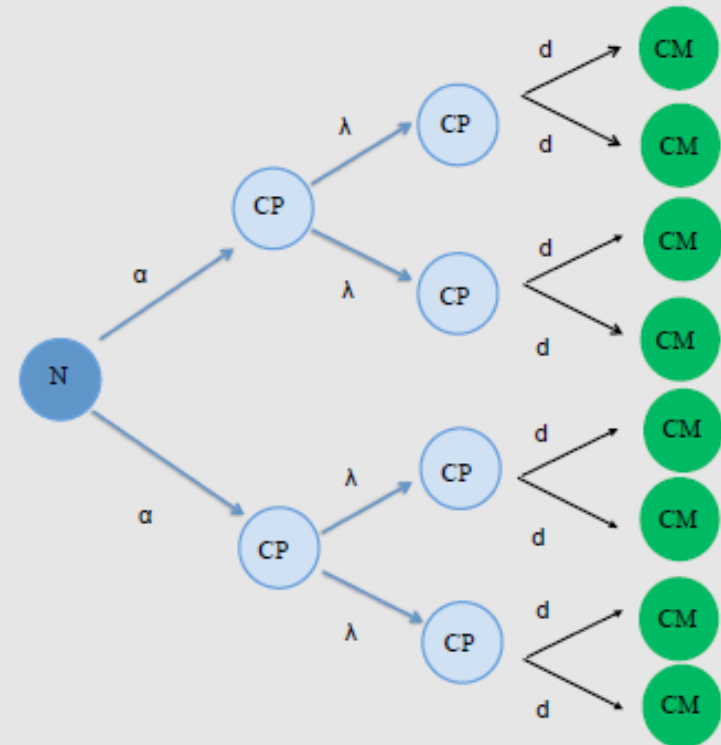
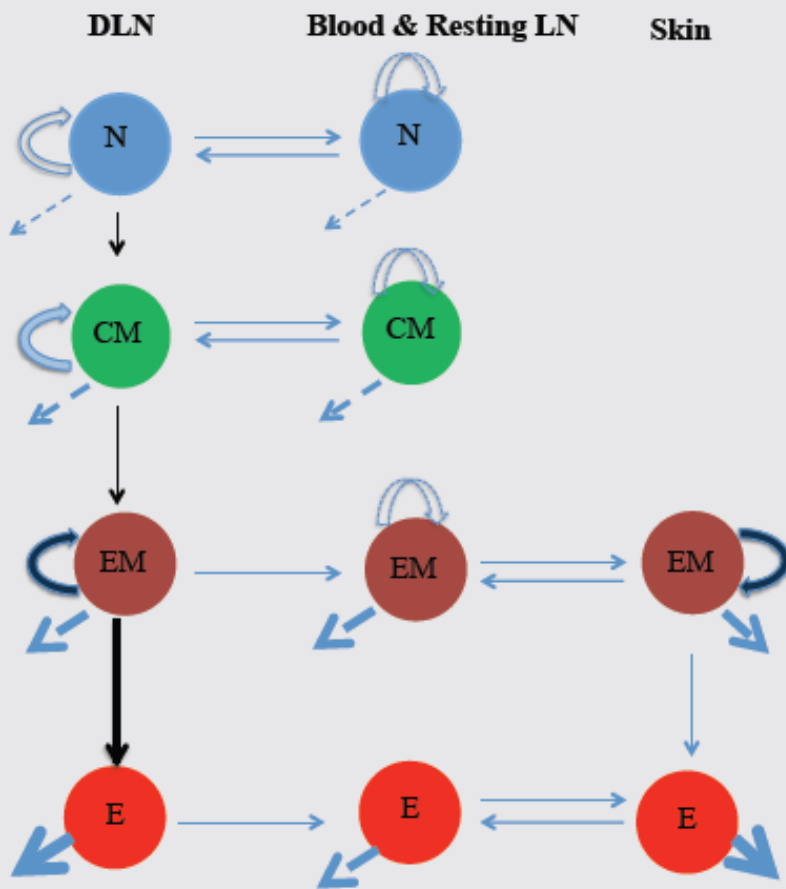
d Asymmetric cell fate model



- Experiments tracking T cell fates have generated a range of hypotheses on T cell differentiation
- Need to select a differentiation mechanism despite uncertainty to predict the number of CD8⁺ memory T cells following sensitizer exposure
- Currently building CD8⁺ T cell models based upon both decreasing-potential (Leeds) & asymmetric-division (Unilever) to explore the impact of each mechanism on predicted T cell response



CD8⁺ T cell mathematical model



- α – rate of contact between naive T cells and APCs in the lymph node.
- λ – rate of proliferation during the clonal expansion.
- d – rate of differentiation.

Heterogeneity: decreasing potential model

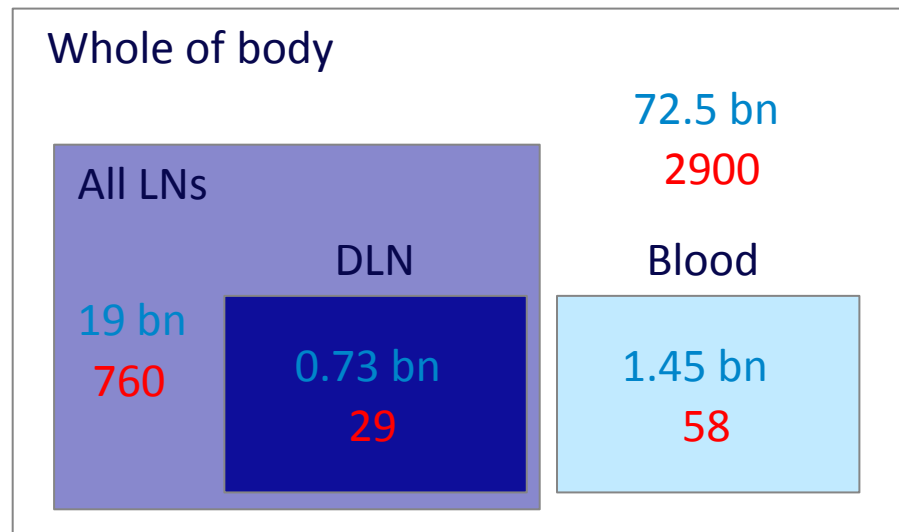


STARTING T CELL POPULATION SIZE



- » Assume no antigen specific effector or memory CD8⁺ T cells at the start in an unexposed individual
- » Estimate number of naïve antigen specific CD8⁺ T cells in DLN & blood
- » Assume exposure to skin on the arm
 - » 25 draining lymph nodes (DLN) in axilla out of 650 in total
 - » Consider a single TCR
 - » One in 25 million naïve T cells are antigen specific

All TCRs
Ag specific (1 TCR)



MODELLING PROGRAMMED T CELL PROLIFERATION



- Following activation, CD8+ T cell proliferation continues independently of further antigenic stimulation
- Going through 7-19 generations (Kaeck & Ahmed, 2001; Badovinac *et al*, 2007) to develop effector and memory populations
- No human data available for proliferation rates
- Obtain proliferation rates from mouse models (e.g. Yoon *et al*, 2010: draining lymph node response to influenza virus infection)

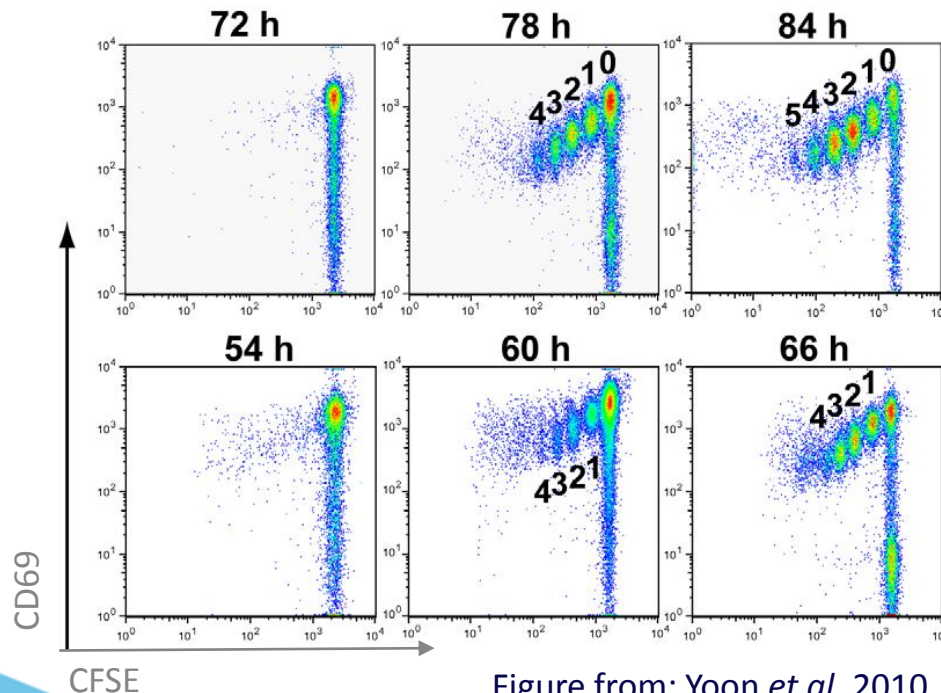
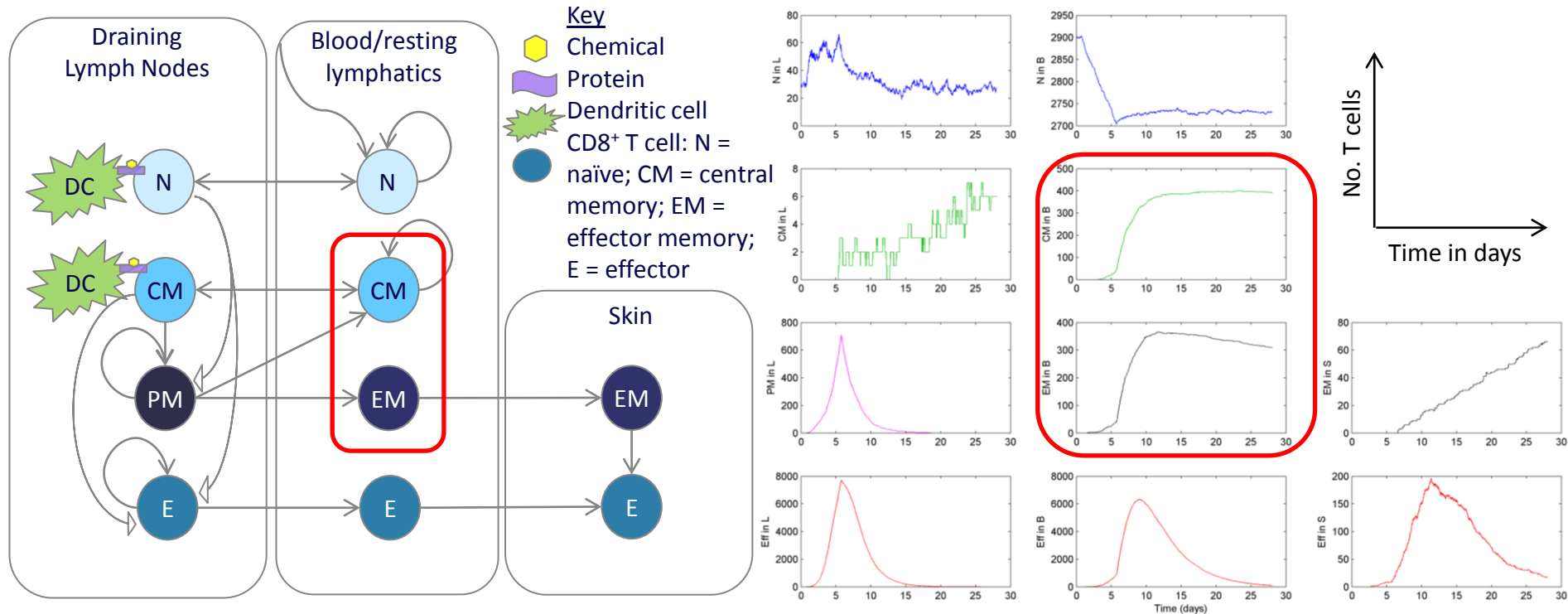


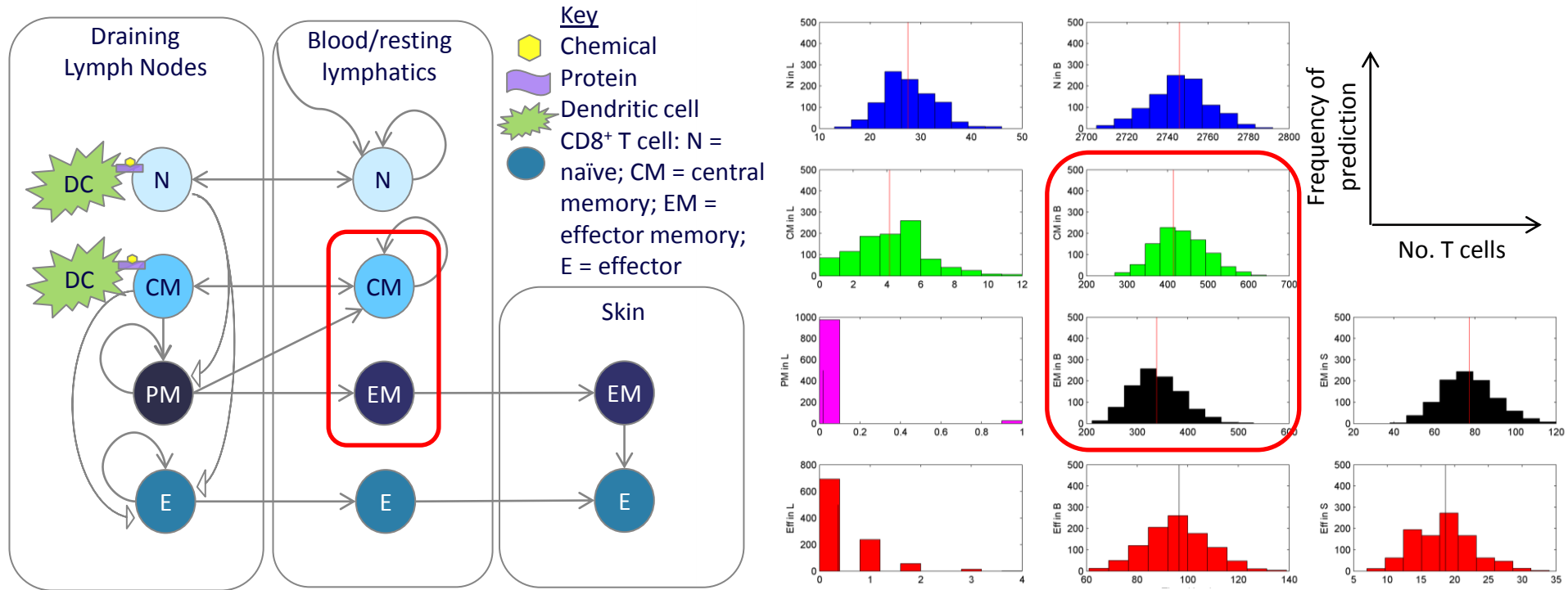
Figure from: Yoon *et al*, 2010, *PLOS One* 5 (11) e15423

CD8⁺ T CELL MODEL PREDICTIONS: 5 DAY ANTIGEN EXPOSURE IN LYMPH NODE, 1X MODEL ITERATION



- Combine the parameters and processes together
- Simulate single exposure to chemical and track response for one month

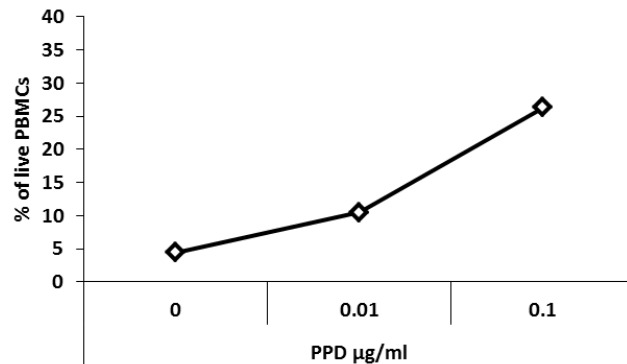
CD8⁺ T CELL MODEL PREDICTIONS: 5 DAY ANTIGEN EXPOSURE IN LYMPH NODE, 1000X MODEL ITERATIONS



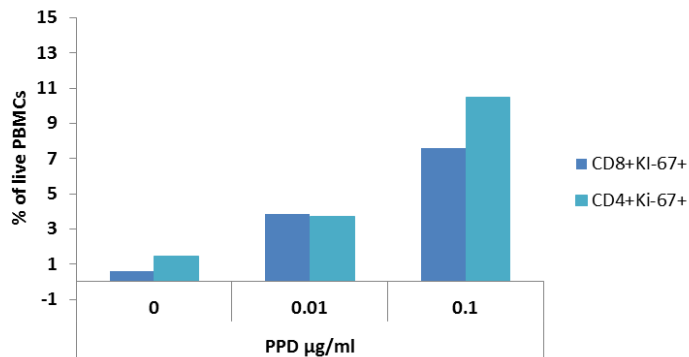
- Combine the parameters and processes together
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Characterising human T lymphocyte responses to chemical allergen *p*-phenylenediamine (PPD)

Total Ki-67 expression



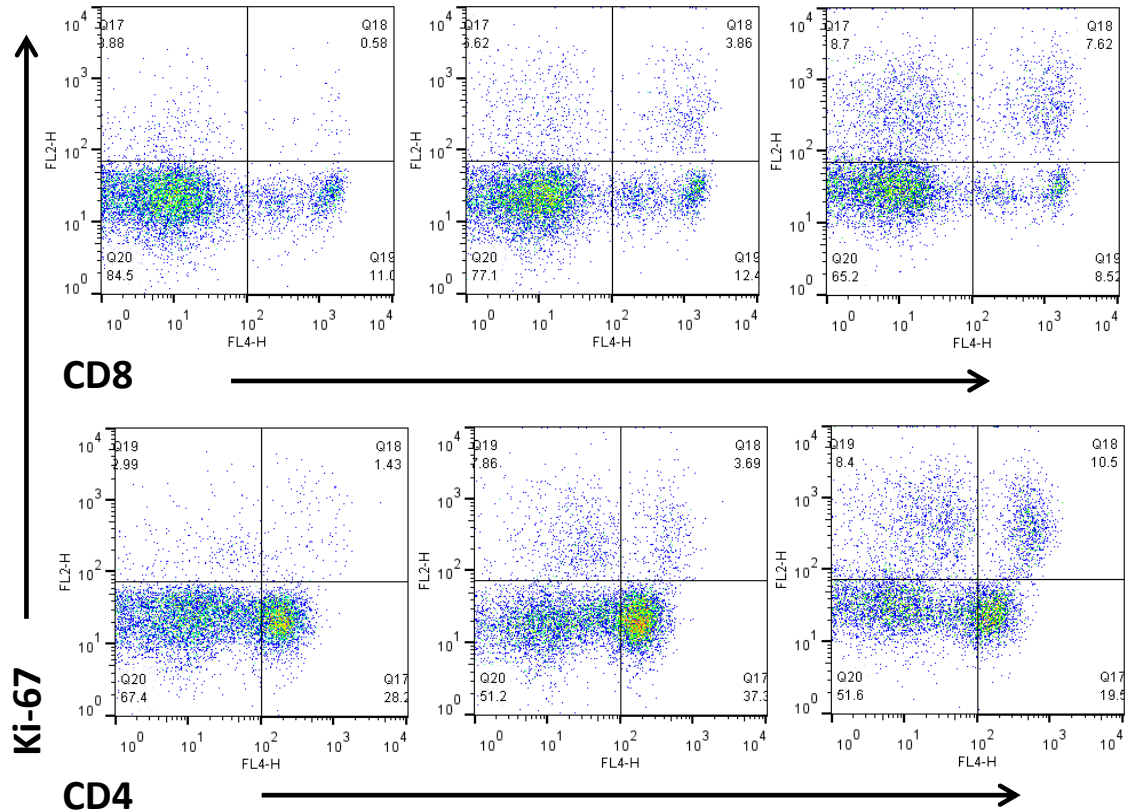
Proliferating T cell subsets



0µg/ml PPD

0.01

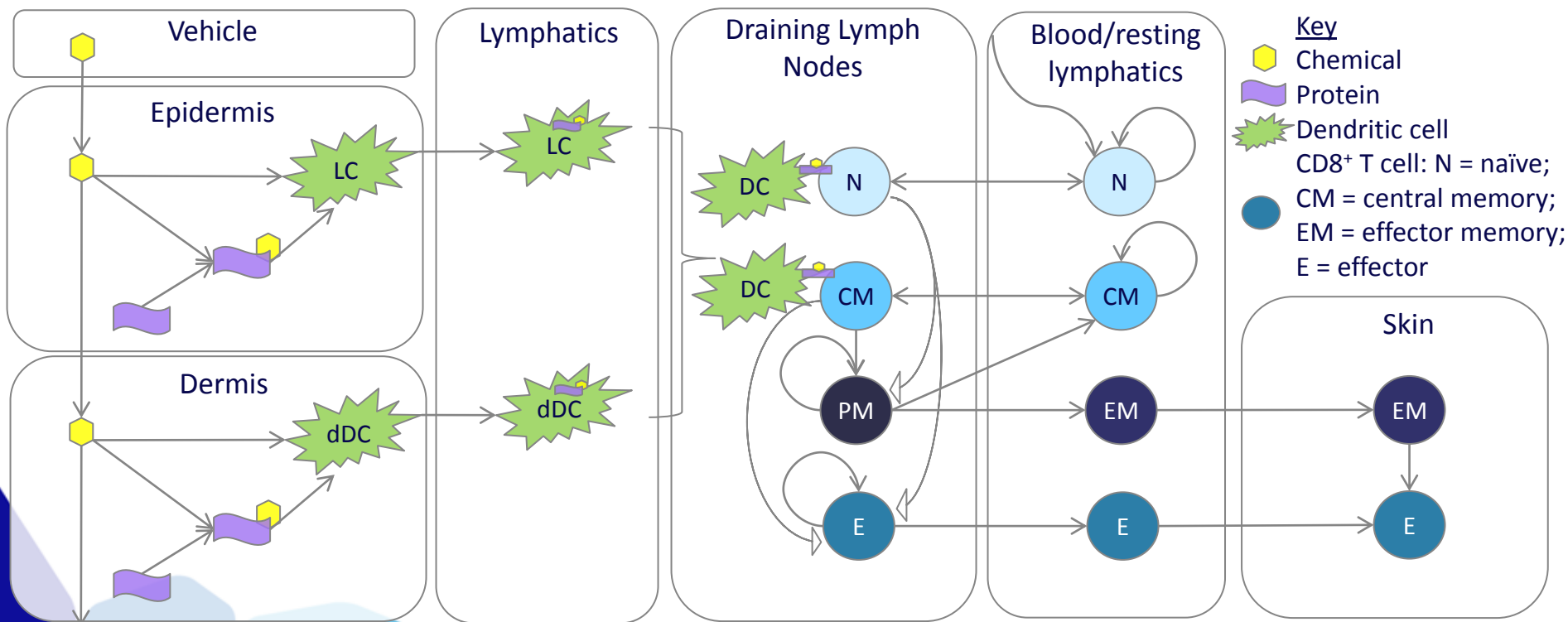
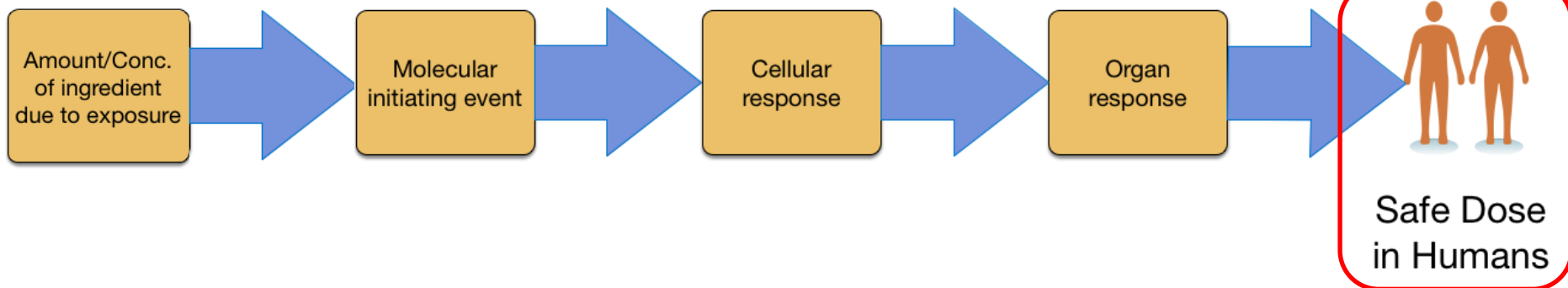
0.1



Allergen driven proliferation of total lymphocytes and individual T cell subsets measured by intracellular Ki-67 expression.

Rebecca Dearman,
Amy Popple, Ian Kimber &
Jason Williams

DEVELOP A MATHEMATICAL MODEL OF ALLERGIC CONTACT DERMATITIS TO ENABLE RISK ASSESSMENT DECISION-MAKING FOR NEW CHEMICALS



PATHWAYS-BASED RISK ASSESSMENT FOR SKIN SENSITISATION: APPLICATION OF MATHEMATICAL MODELLING

1. Skin Penetration

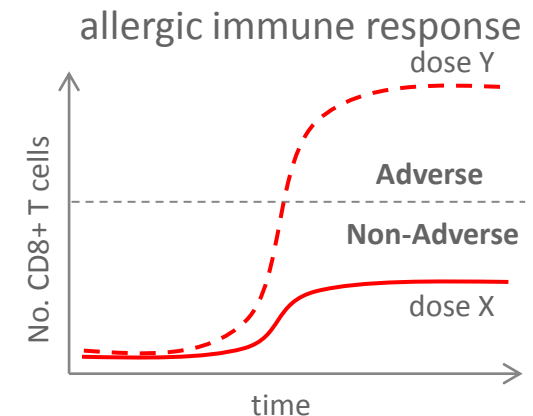
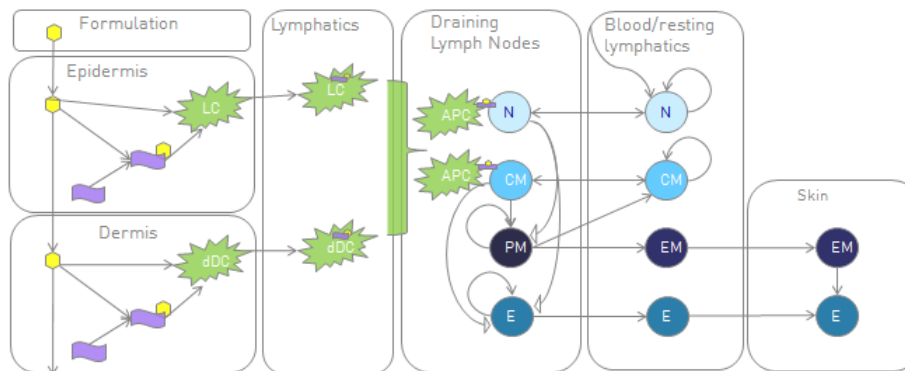
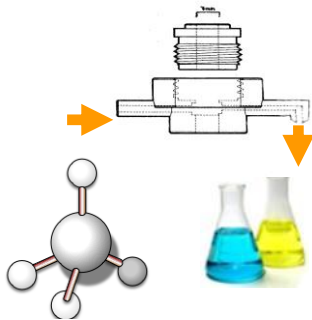
2. Electrophilic substance: directly or via auto-oxidation or metabolism

3-4. Haptenation: covalent modification of epidermal proteins

5-6. Activation of epidermal keratinocytes & Dendritic cells

7. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells

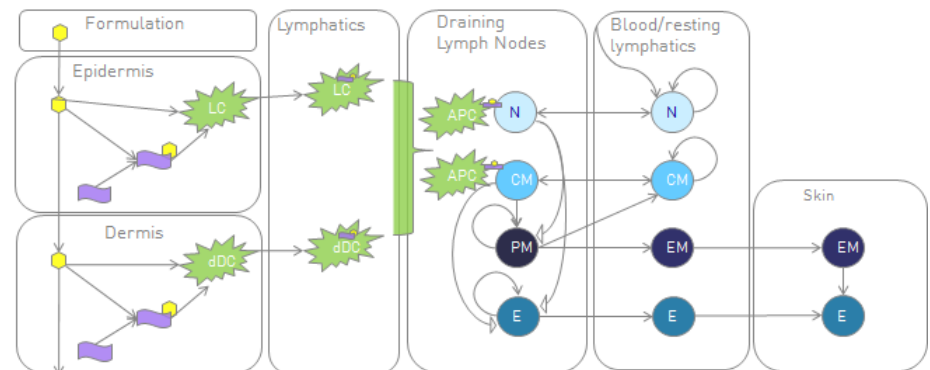
8-11. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death



1. Generate skin bioavailability & haptenation data as model input parameters
2. Use linked mathematical models to predict human allergic immune response
3. Apply human immune response model prediction for risk assessment decision
4. If exposure predicted to be non-adverse, verify prediction using clinical data

NEXT STEPS: CHALLENGES AHEAD

- Broadening current model scope to include:
 - CD4⁺ T helper & regulatory T cell responses
 - sensitiser-induced inflammation in skin – induction & elicitation
 - impact of varying frequency & surface area of sensitiser exposure
 - impact of varying formulation (vehicle)
- Using experimental & clinical data to inform & benchmark initial model predictions



ACKNOWLEDGEMENTS

Unilever



Richard Cubberley, Seraya Dhadra, Michael Davies, Nikki Gellatly, Stephen Glavin, Todd Gouin, Sandrine Jacquoillet, Cameron MacKay, Craig Moore, Ruth Pendlington, Juliette Pickles, Ouarda Saib, David Sheffield, Richard Stark, Vicki Summerfield & Sam Windebank



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